PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A23K 1/165, 1/175, 1/18

A1

(11) International Publication Number: WO 99/49740

(43) International Publication Date: 7 October 1999 (07.10.99)

(21) International Application Number: PCT/EP99/02311

(22) International Filing Date: 1 April 1999 (01.04.99)

(30) Priority Data:
98201029.0 1 April 1998 (01.04.98) EP

(71) Applicant *(for all designated States except US)*: DSM N.V. [NL/NL]; Het Overloon 1, NL–6411 TE Heerlen (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BEUDEKER, Robert, Franciscus [NL/NL]; Boomkwekerij 31, NL–2635 KC Den Hoorn (NL). KIES, Arie, Karst [NL/NL]; Freule van Dorthsingel 57, NL–2642 AC Pijnacker (NL). HOF, Jan, Bart [NL/NL]; Zoudtlandseweg 9, NL–4838 GR Breda (NL).

(74) Agent: VAN HEUVEL, Margaretha; DSM Patents and Trademarks, Office Delft (994–0760), P.O. Box 1, Wateringseweg 1, NL–2600 MA Delft (NL).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

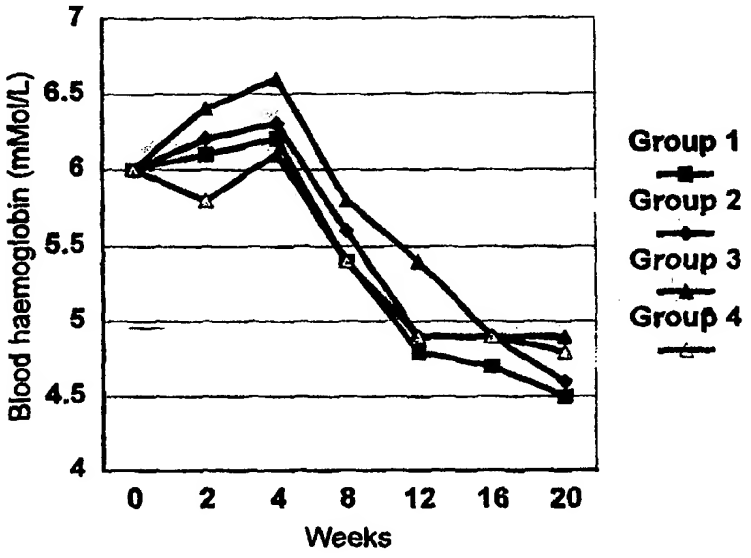
With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: APPLICATION OF PHYTASE IN FEED HAVING LOW CONTENT OF PHYTATE

(57) Abstract

The present invention relates to the application of the enzyme phytase in feed having a low-phytate content. The invention discloses methods of feeding a low-phytate diet supplemented with a phytase. Feeding an animal a low-phytate diet comprising a phytase improves the mineral status of the animal and results in improved zootechnical results such as improved growth rate and feed conversion ratio. The invention also discloses a low-phytate feed for animals supplemented with a phytase as well as methods for the preparation of such feed.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

APPLICATION OF PHYTASE IN FEED HAVING LOW CONTENT OF PHYTATE

Field of the invention

The present invention relates to the application of the enzyme phytase in feed having a low content of phytate.

Background of the invention

The diets of ruminants vary considerably in different husbandry systems, but also at different ages. In extensive husbandry systems, typically animals are fed with roughages, like grass, hay, grass silage and/or maize silage. Some additional feeds or feedstuffs may be offered, likewise a vitamin and/or mineral mixture. In such husbandry systems, young animals typically suckle with their mother. In more intensive husbandry systems, young animals, e.g. calves, are often offered a milkreplacer, since the produced milk of the cows is used for human consumption, or for processing to products for human consumption, like butter and cheese. Veal calves are usually mainly offered a milkreplacer. Some other feeds or feedstuffs, like hay, corn, or a special mixed concentrate may be offered additionally. Often these feedstuffs require a low level of available iron, when the aim is to produce white veal calf meat. For calves aimed for rose meat production, the feeds preferably contain some available iron. Older animals, like dairy cows, beef cattle, sheep and deer, typically receive rations containing roughages, e.g. grass, hay, grass silage and/or maize silage, and more concentrated feedstuffs, e.g. mixed concentrates (usually produced in a feed mill), cereals,

vegetable (by-) products and/or brewers grains. Additionally, a mineral and/or vitamin mixture may be offered.

Phosphorus is an essential element for growth of all organisms. In livestock production, feed must be supplemented with inorganic phosphorus in order to obtain a good growth performance of monogastric animals (e.g. pigs, poultry and fish).

Phytate occurs as a storage phosphorus source in virtually all feed substances originating from plants (for a review see: Phytic acid, chemistry and applications, E. Graf (ed), Pilatus Press; Minneapolis, MN, USA (1986). Phytate comprises 1-3% of all nuts, cereals, legumes, oil seeds, spores and pollen. Complex salts of phytic acid are termed phytin. Phytic acid is considered to be an anti-nutritional factor since it chelates minerals such as calcium, zinc, magnesium, iron and may also react with proteins, thereby decreasing the bioavailability of protein and nutritionally important minerals.

The use of phytase in the nutrition of monogastric animals (e.g. pigs, poultry and fish) has become widespread thanks to the availability of microbial phytases at an affordable cost. Cloning and overexpression of microbial phytase has resulted in a dramatic decrease in cost price of the product enabling the commercialisation of this enzyme in the nutrition of monogastric animals.

In contrast, typically no inorganic phosphate needs to be added to the feed stuffs of older ruminants. Micro-organisms present in the rumen are thought to produce sufficient amounts of enzymes which catalyse the conversion of phytate (myo-inositolhexakis-phosphate) to inositol and inorganic phosphate.

In Europe and the USA, the feeds wherein phytase advantageously is applied typically have a phytate-phosphorus content of 0.25 to 0.35%.

We now demonstrate that the addition of phytase to feed having a low phytate content such as feed for young animals (ruminants or

non-ruminants) results in a marked improvement in daily gain and feed conversion ratio.

Description of the Figures

Figure 1. Contents of zinc in blood plasma as a function of time during the fattening period, for each of the four groups with a different diet composition: Group 1, $ZnSO_4$ and phytase; Group 2, only $ZnSO_4$; Group 3, only phytase; Group 4, no additions.

Figure 2. Blood haemoglobin levels as a function of time during the fattening period, for each of the four groups with a different diet composition: Group 1, $ZnSO_4$ and phytase; Group 2, only $ZnSO_4$; Group 3, only phytase; Group 4, no additions.

Description of the invention

The present invention relates to the application of the enzyme phytase in feed with a low phytate content. In particular, the present invention relates to the application of the enzyme phytase in the feeding of animals which typically are fed low-phytate diets, such as poultry or young animals.

According to an aspect of the invention there is provided a method of feeding an animal, wherein the animal is fed a low-phytate diet supplemented with a phytase. According to another aspect of the invention there is provided a method of feeding a young animal a low-phytate diet supplemented with a phytase which is applied as part of a method of breeding and/or keeping an animal.

One would not expect any beneficial effect of phytase when applied in feeds with a low content of phytate. The present invention surprisingly shows that the addition of exogenous phytase to low-phytate feeds results in a marked improvement in zootechnical results, such as average daily gain (i.e. growth rate) and feed conversion, in animals fed such phytase-containing low-phytate feeds.

Accordingly, one aspect of the invention provides a method of improving the zootechnical results of an animal, wherein the method comprises feeding the animal a low-phytate feed supplemented with a phytase. This improvement of the zootechnical results of animals fed with a low-phytate diet supplemented with a phytase may be apparent in any animal fed with a low-phytate diet. In particular, an improvement of the zootechnical results was measured in young animals.

Contents of zinc in blood plasma and haemoglobin levels are also significantly increased as a consequence of phytase addition to low-phytate feed whereas levels of calcium, iron, magnesium and copper remain unaffected. The mere addition of a mineral such as zinc to the feed does improve the mineral status of the animals but only slightly improves the zootechnical results such as growth rate and feed conversion ratio.

Accordingly, yet another aspect of the invention provides a method of improving the mineral status of animals, wherein the method comprises feeding the animal a low-phytate diet supplemented with a phytase. In a preferred method according to the invention, the low-phytate diet fed to the animal further comprises zinc in addition to a phytase. Preferably 0.005 - 0.2% (w/w) zinc is included in the diet, more preferably 0.01 - 0.03% is included in the diet.

The term "zootechnical results" is herein understood to mean animal performance in terms of daily gain (growth rate), feed conversion ratio (FCR), daily milk production and the like. FCR is defined as the amount of feed (kg) required per kg of growth of the animal.

Throughout this invention, a low-phytate feed or diet is defined as a feed or diet comprising phytate-phosphorus (phytate-P) in a concentration ranging from 0.01 to 0.2% (w/w), preferably from 0.01 to 0.15%, and more preferably from 0.01 to 0.1%. Most preferably, low-phytate feeds have a phytate-P content of 0.01 to 0.05%, which is up to ten times lower than the feed of an older animal.

Phytate can be determined as phytic acid, according to the method of e.g. Oshima et al., 1964 (Oshima, M., T.G.Taylor and A.Williams, Biochemical Journal 92, 42-46). Phytate-P is calculated as phytic acid * 0.29, since phytic acid contains approximately 29% phosphorus.

For the purpose of this invention, young animals are defined as farmed animals which are in their growing and/or fattening period and/or which would still suckle with their mother in natural conditions and/or which receive a feed product specifically adapted for young animals, especially young ruminants, as defined hereinafter. Young animals can be young ruminants such as calves, heifers, lamb, deer calves or goats. Young animals can also be young non-ruminants, such as piglets, broilers or pullets.

A phytase is herein defined as an enzyme which is a phosphatase capable of liberating at least one inorganic phosphate from a myo-inositol phosphate. A typical example of a phytase is a myo-inositol-hexakisphophate-3- phosphohydrolase (E.C. 3.1.3.8).

The phytase to be applied in the methods and products according to the invention is not present as a natural constituent of any of the feed stuffs in the animal diet but rather is a supplement to feed stuffs or the diet in general. This means that the phytase applied in the methods and products of the invention differs from any of the natural phytases that might be present in the feed stuffs with respect to the amount of phytase activity and/or the nature of the phytase, i.e. the organism from which the phytase is obtainable and/or the structure and/or biochemical properties of the phytase molecule. Thus, the phytase applied in the methods and

products of the invention is a supplement to the low-phytate feed of the animal. The phytase supplement can be an exogenously added phytase, which can be produced by fermentation of microbial host cells expressing the phytase. Cloning and overexpression of microbial phytases has been described in detail in EP-A-0 420 358. Another possibility of supplementing a low phytate feed with a phytase is to add phytase-containing transgenic plant material, preferable transgenic seed, which has been genetically engineered so as to express (or overexpress) a phytase as described in detail in EP-A-0 449 375.

The phytase to be applied in the methods of the invention is preferably a phytase with an acidic pH optimum, i.e. with an optimum at a pH less than 7.0, preferably less than 6.0. Preferably the phytase is obtainable from plants or micro-organisms. The microbial phytase is preferably obtainable from a fungus, more preferably from a fungus of the genera *Aspergillus* and *Thermomyces*. Most preferably the microbial phytase is obtainable from a black *Aspergillus* that belongs to the *Aspergillus niger* Group as defined by Raper and Fennell (1965, In: The Genus *Aspergillus*, The Williams & Wilkins Company, Baltimore, pp 234-344), such as *Aspergillus niger*, *Aspergillus ficuum* and *Aspergillus awamori*.

In the methods and products according to the invention the phytase is preferably added to the low-phytate feed of the animal in an amount exceeding the amount of phytase activity naturally present in any of the feed stuffs ordinarily used in the preparation of animal feed. The activity level of the phytase supplemented to the low-phytate feed is preferably at least 10 FTU (for definition see Example 1) per kg of low-phytate feed, more preferably at least 20 FTU per kg of low-phytate feed, more preferably at least 50 FTU per kg of low-phytate feed, more preferably at least 100 FTU per kg of low-phytate feed, more preferably at least 200 FTU per kg of low-phytate feed. Usually the activity level will be less than 10,000 FTU per kg of low-phytate feed, preferably less than 5000 FTU

per kg of low-phytate feed, more preferable less than 2500 FTU per kg of low-phytate feed.

According to another aspect of the invention there is provided a method for preparing a phytase-containing low-phytate feed for animals, wherein feed stuffs are mixed with a phytase, and optionally zinc. The phytase may be mixed with the feed stuffs in dry form, e.g. as an enzyme containing granulate, or in liquid form, e.g. in the form of a stabilised liquid concentrate. In a preferred method the phytase is mixed with the feed stuffs as part of a premix which may contain other feed additives such as other enzymes, vitamins, minerals. For animals receiving (mainly) a milkreplacer, this feed would be an optional way to offer phytase to the animal.

A product according to the invention provides a low-phytate feed comprising feed components specifically adapted for the said animal supplemented with a phytase. In one embodiment of the invention, a low-phytate feed may be provided by mixing feed components with a high phytate content with feed components with a low phytate content. In another embodiment of the invention, a low-phytate feed may be provided by including a plant source in the feed which is genetically modified and/or obtained by classical selection techniques to contain a lower amount of phytate than the amount which is present in the unmodified and/or parental plant. For instance, the use of low-phytate corn or soy variants for feed preparation may provide feeds with a phytate-P content of about 0.2%.

Another product according to the invention provides a low-phytate feed specifically adapted for young animals, especially young ruminants, supplemented with a phytase. For instance, a feed for veal calves in the growing and fattening period will usually consist of mixtures of skim milk powder (and other milk products) and milk substitutes (milk replacers) of vegetable origin such as soybean isolates, soybean concentrates and wheat as well as animal fat and vitamins and minerals. Some other feeds

or feedstuffs, like hay, corn, or a special mixed concentrate may be offered additionally.

Accordingly, a low-phytate feed specifically adapted for young animals, especially young ruminants, in the growing and fattening period is a milk replacer which, in addition to phytase, preferably comprises less than 60% (w/w solid matter) skim milk powder, more preferably less than 40%, more preferably less than 30%, more preferably less than 20%, more preferably less than 10%, and most preferably 0%. Preferably the feed for young animals in the growing and fattening period comprises at least 5% of milk substitutes of a vegetable origin, more preferably at least 25%, most preferably at least 50%.

Some milk replacer produced also is used for monogastric animals, especially for piglets.

Thus, according to one aspect of the invention, there is provided a method for feeding an animal, such as poultry, a low-phytate feed supplemented with a phytase. Preferably, said low-phytate feed comprises a genetically modified or classically obtained low-phytate corn or soy source, and/or alternatively, another low-phytate plant source.

According to a preferred aspect of the invention there is provided a method of feeding a young animal in the growing and/or fattening period, such as a young ruminant, a low-phytate diet supplemented with a phytase. The diet comprises milk products, of which the amount of skim milk powder preferably is as low as possible, and milk substitutes as described hereinabove and is supplemented with a phytase.

The invention is also directed to the non-therapeutic use of a phytase in any of the methods according to the invention such e.g. a method of feeding an animal a low phytate feed, the use of a phytase for the improvement of zootechnical results in breeding and/or keeping of said animal, as well as to the use of a phytase for the improvement of the mineral status of said animal.

The following Examples serve to illustrate the invention and are by no means intended to limit the invention in any way.

Example 1

Example 1

Effects of microbial phytase on growth and feed conversion ratio up to 177 kg of live weight

Trials are carried out with 4 groups of 19 piebald bull calves. After a starting period of 6 weeks animals received the following diets during a 20 week fattening period: feed was either supplemented with microbial phytase (NATUPHOS® 5000, Gist-brocades, Delft, the Netherlands, obtainable from BASF, Ludwighshafen, Germany), $ZnSO_4$ or both. Group 4 served as a negative control. Microbial phytase was supplemented at a dose of 500 FTU (phytase units) per kg of feed. The analytical method for determining microbial phytase activity and the definition of a phytase unit has been published by Engelen et al. (Journal of AOAC International 77 (3): 760-764 (1994).

Based on the diet composition of Table 1 the contents of various nutrients have been calculated as follows for all diets:

Nutrient (g/kg): Crude protein (185.2), Crude Fat (229.9), Crude Fiber (0.6), Moisture (35.9), Ash (65.3), Nitrogen free extract (451.1), Lactose (35.1), Starch (83.7), Dairy protein (51.8), non-dairy protein (13.5), Lysine (17.9), Methionine (7.5), Met + Cys (10.4), Threonine (8.4), Tryptophan (2.2), Isoleucine (8.4), Calcium (8), Phosphorus (5.7), Phytate-P (0.5), Iron (23 mg/kg). ME = 4471.1 kcal/kg.

Table 2 shows the average feed intake, growth and feed conversion ratio (FCR) calculated therefrom for each of the four groups.

Table 1. Diet composition for Groups 1-4 during the fattening period

	Group 1	Group 2	Group 3	Group 4
Raw material	%	%	%	%
Whey powder	51.8	51.8	51.8	51.8
Soy protein isolate	12.5	12.5	12.5	12.5
Wheat gluten	11	11	11	11
Animal fat	8	8	8	8
Lard	7.5	7.5	7.5	7.5
Coconut oil	5	5	5	5
Lecithin	1	1	1	1
Emulsifier (Berol)	0.65	0.65	0.65	0.65
Dicalcium phosphate	0.2	0.2	0.2	0.2
Calcium formiate	1.25	1.25	1.25	1.25
DL-methionine	0.05	0.05	0.05	0.05
Vitamin premix	1	1	1	1
Zinc sulphate	0.05	0.05	0	0
Phytase (FTU/kg)	500	0	500	0

Table 2. Feed intake, growth and feed conversion ratio of Groups 1-4 during the fattening period

	Group 1	Group 2	Group 3	Group 4
Feed intake (kg)	327.5	328.5	335.1	323
Growth (kg)	164.4	168.4	177.2	161.2
FCR (kg/kg)	1.99	1.95	1.89	2

Best results were obtained for group 3 grown on feed containing supplemental phytase and no additional $ZnSO_4$.

Example 2

Effects of microbial phytase on zootechnical parameters up to 267 kg of live weight.

A similar experiment as described in Example 1 was conducted with older animals. This is relevant for practice since results obtained may vary as a function of body weight and age.

The study was conducted with veal calves with a live weight of 130-150 kg at the start of the experimental period. In total 24 male black-and-white veal calves (Holstein-Friesian) were used for the experiment.

On day 0 all animals were weighed and divided among three experimental groups in such a way, that the groups were as homogenuous as possible. Allocation was based on body weight and haemoglobin content of the blood.

The animals were housed individually under conventional conditions in wooden boxes (75*175 cm) with slatted floors.

After allocation, the animals were changed from a commercial milk replacer to the experimental diet plus premix. The composition of the diet was as described in Table 3.

Microbial phytase was supplemented as NATUPHOS® 5000 (Gist-brocades, Delft, the Netherlands, obtainable from BASF, Ludwigshafen, Germany) in the diets.

During the experiment, the animals were fed according to the schedule shown in Table 4. Animals were fed in the morning and in the afternoon. The milk replacer including the phytase was dissolved in hot water (60-70°C), mixed for 3-5 minutes, followed by addition of cold water while mixing until the required amount of milk with a temperature of 40-41°C was prepared. The animals were not allowed to drink extra water.

Table 3: Diet composition for Groups 1-3 during the fattening period

	Group 1	Group 2	Group 3
Raw material	%	%	%
Whey powder	41.7	41.7	41.7
Whey powder, delactosed	14	14	14
Whey protein	10	10	10
Soy protein (HP 100)	10	10	10
Pregelatinized starch	2.5	2.5	2.5
Fat*	20.15	20.15	20.15
Methionine	0.35	0.35	0.35
Lysine	0.75	0.75	0.75
Zinc sulphate	0.02	0.02	0.02
Premix (%)	0.5	0.5	0.5
Phytase (FTU/kg)	< 20	1,000	10,000

* Fat composition: 37% tallow, 40% lard, 15% coconut fat, 5% lecithin and 3% emulsifier.

The above diet contained 0.04% phytate-P.

Table 4: Feeding schedule

Day	Kg milk per calf per feeding	Concentration (g powder per kg milk)	Intake powder (g per animal per feeding)
0-7	8.5	130	1,105
7-14	8.5	135	1,148
14-21	8.5	140	1,190
21-28	8.5	145	1,233
28-35	8.5	150	1,275
35-42	8.5	155	1,318
42-49	8.5	160	1,360
49-56	8.5	165	1,403
56-63	8.5	170	1,445
63-77	8.5	175	1,488
77-90	8.5	180	1,530
91	8.5	130	1,105

The animals were weighed before allocation and at 4,8,12 and 13 weeks after allocation with a precision of 0.2 kg. Feed intake was registered per animal. With these figures and weight gain results, feed conversion ratio was calculated as kg feed per kg weight gain.

Body weight (kg) at different times is shown in Table 5.

Table 5

Days experimental period	Negative control group	Group with 1,000 FTU/kg	Group with 10,000 FTU/kg
0	140.8	140.8	140.9
28	176.9	180	181.3
56	213.7	217.1	218
84	244	252	254.1
90	251	259.1	262.5
91	252.9	261.4	265.4

Experimental groups receiving diets comprising phytase show a substantially higher daily gain in bodyweight than the negative control group.

Cumulative feed conversion ratio (kg feed/kg body weight gain) during the different periods for the three different groups is shown in Table 6.

Table 6

Days experimental period	Negative control group	Group with 1,000 FTU/kg	Group with 10,000 FTU/kg
0-28	1.83	1.69	1.64
0-56	1.93	1.85	1.85
0-84	2.16	1.99	1.99
0-90	2.2	2.04	2.02
0-91	2.19	2.02	1.99

Experimental groups receiving diets containing supplemented microbial phytase show a considerable improvement in feed conversion ratio.

Example 3

Effects of microbial phytase on mineral status

Animals were grown as detailed in Example 1. Blood samples were taken at the start of the fattening period and 2, 4, 8, 12, 16 and 20 weeks thereafter. Blood was analysed for the content of haemoglobin, iron, calcium, magnesium, copper, and zinc following methods known to people skilled in the art. Contents of calcium, magnesium, copper and iron were the same for all test groups. The content of iron in blood plasma decreased markedly during this period but did not differ significantly between the groups. There appeared to be a significant difference in zinc contents in blood plasma between the groups as shown in Figure 1.

Group 1 receiving elevated levels of $ZnSO_4$ and phytase in the diet showed highest levels of zinc in blood plasma. Group 3, receiving phytase only and no added $ZnSO_4$, showed intermediate zinc levels in blood plasma. The same is true for group 2 receiving supplemental zinc in the diet and no phytase. The negative control group showed very low levels of zinc in blood plasma.

It is concluded that it is possible to improve the mineral status of the animal by either supplementing the diet with $ZnSO_4$, by the addition of phytase or by the combination of both.

The effects of age and diet composition on blood haemoglobin levels are shown in Figure 2. The increased levels of haemoglobin in animals receiving diets containing phytase and no added minerals (Group 3) is another indication of a positive effect of phytase on the performance of the animals.

CLAIMS

1. A method of feeding an animal a feed supplemented with a phytase, wherein said feed is a low-phytate feed having a content of phytate-P in a concentration ranging from 0.01 to 0.2% (w/w), preferably from 0.01 to 0.15%, more preferably from 0.01 to 0.1%, most preferably from 0.01 to 0.05%.

2. A method of breeding and/or keeping an animal, comprising feeding the animal according to the method of claim 1.

3. A method of improving the zootechnical results of an animal, comprising feeding the animal according to the method of claim 1.

4. A method of improving the mineral status of an animal, comprising feeding the animal according to the method of claim 1.

5. A method according to any one of the preceding claims, wherein the feed further comprises zinc.

6. A method according to any one of the preceding claims, wherein the phytase is supplemented at an activity level of at least 10 FTU per kg of feed.

7. A method according to any one of the preceding claims, wherein the animal is a young animal.

8. The method according to claim 7, wherein the young animal is a young ruminant selected from the group consisting of calf, heifer, lamb, deer calf and goat.

9. The method according to claim 7, wherein the young animal is a piglet.

10. The method according to claim 7, wherein the young animal is a broiler or pullet.

11. A method according to any one of the preceding claims, wherein the feed is specifically adapted for a young animal.

12. A method for preparing a feed for animals, wherein the method comprises the step of mixing feed stuffs and a phytase.

13. A method according to claim 12, wherein the phytase is mixed with the feed stuffs as part of a premix.

14. A method according to any one of the preceding claims, wherein the phytase is obtainable from a plant or a micro-organism.

15. A method according to any one of the preceding claims, wherein the phytase is obtainable from a fungus, preferably from a fungus of the genus *Aspergillus*.

16. A low-phytate feed for animals having a content of phytate-P in a concentration ranging from 0.01 to 0.2% (w/w), preferably from 0.01 to 0.15%, more preferably from 0.01 to 0.1% and most preferably from 0.01 to 0.05%, comprising feed components specifically adapted for said animals and supplemented with a phytase which does not naturally occur in the feed components.

17. A feed according to claim 16, wherein the phytase is present at an activity level of at least 10 FTU per kg of feed.

18. Use of a phytase for the improvement of zootechnical results in breeding and/or keeping of animals.

19. Use of a phytase for the improvement of the mineral status of animals.

Figure 1.

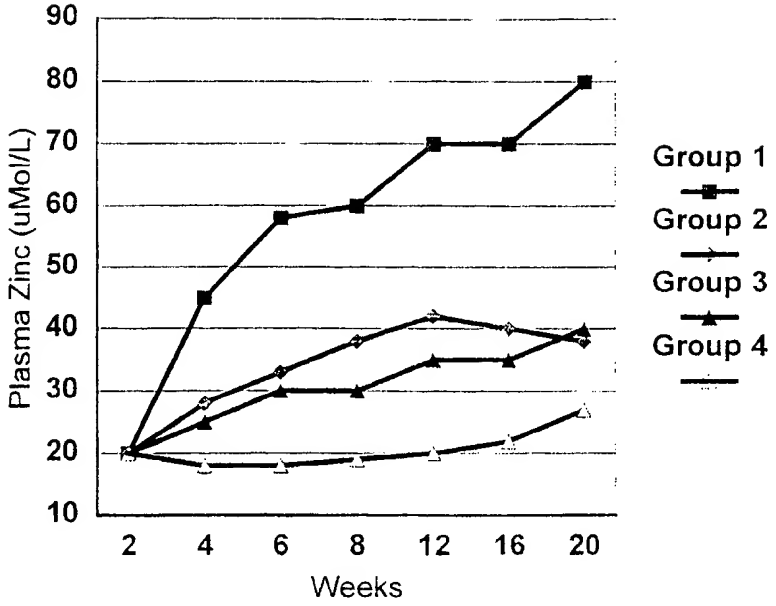
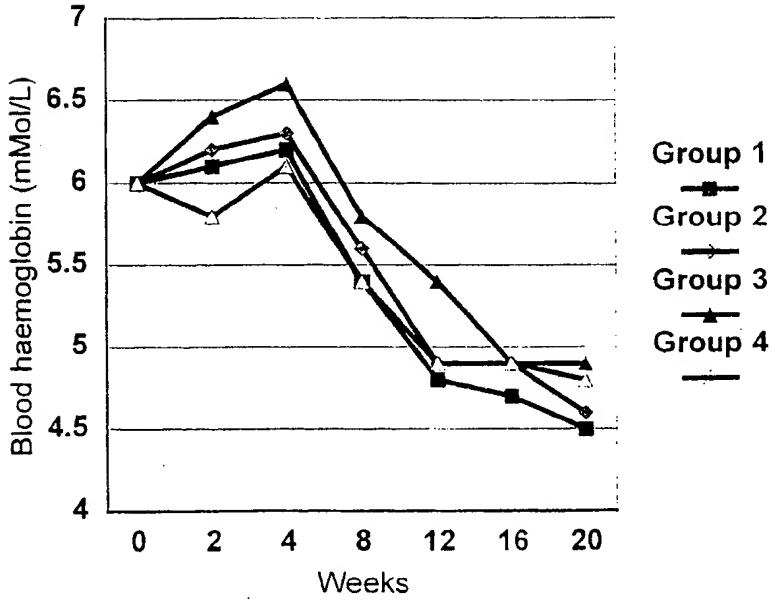


Figure 2.



INTERNATIONAL SEARCH REPORT

Inte .ional Application No
PCT/EP 99/02311

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23K1/165 A23K1/175 A23K1/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 449 375 A (MOGEN INT ;GIST BROCADES NV (NL)) 2 October 1991 (1991-10-02) cited in the application claims 16-18,21	18
A	example 13	1-7, 10-17

X	EP 0 779 037 A (GIST BROCADES NV) 18 June 1997 (1997-06-18) page 4, line 25 - line 41 claims 1-9	18
A		1-17,19

X	WO 97 35017 A (NOVO NORDISK BIOTECH INC) 25 September 1997 (1997-09-25) page 28, line 5 - line 21 claims 1,43,47,48	18
A		1-17,19

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 August 1999	31/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Dekeirel, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. tional Application No
PCT/EP 99/02311

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	GB 2 316 082 A (FINNFEEDS INT LTD) 18 February 1998 (1998-02-18) page 4, paragraph 4 - last paragraph page 5, last paragraph - page 6, paragraph 2 page 10, paragraph 2 - paragraph 3 page 19, paragraph 6 figure 7 claims 1,24-29	18
P,A	WO 99 02668 A (EXSEED GENETICS LLC ;CHANG MING TANG (US); KEELING PETER L (US); W) 21 January 1999 (1999-01-21) page 37, line 13 - page 38, line 4 claims 1,3,12,13,15	1,16
A	WO 97 48812 A (UNITED KINGDOM GOVERNMENT ;YANKE LINDSEY JAY (CA); CHENG KUO JOAN) 24 December 1997 (1997-12-24) page 5, line 18 - page 6, line 17 page 19, line 12 - line 23; claim 6 claims 36-50,58-67	1-3,6-8, 11-14, 16-18
A	GB 1 514 156 A (INTERCHEMIE AG) 14 June 1978 (1978-06-14) page 2, line 46 - line 57 example 2	1,4,5,7, 8

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. ional Application No
PCT/EP 99/02311

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0449375 A	02-10-1991	AU 649447 B	26-05-1994
		AU 7765691 A	21-10-1991
		AU 632941 B	14-01-1993
		AU 7776691 A	21-10-1991
		CA 2054762 A	24-09-1991
		CA 2056396 A	24-09-1991
		EP 0449376 A	02-10-1991
		HU 215164 B	28-10-1998
		HU 215260 B	30-11-1998
		IL 97645 A	18-03-1997
		JP 6501838 T	03-03-1994
		JP 6502296 T	17-03-1994
		WO 9114782 A	03-10-1991
		WO 9114772 A	03-10-1991
		PT 97110 A,B	29-11-1991
		PT 97111 A	31-12-1991
		US 5543576 A	06-08-1996
		US 5714474 A	03-02-1998
		US 5593963 A	14-01-1997
		US 5770413 A	23-06-1998
EP 0779037 A	18-06-1997	EP 0420358 A	03-04-1991
		AT 180014 T	15-05-1999
		AU 636673 B	06-05-1993
		AU 6501190 A	28-04-1991
		BG 60108 A	15-10-1993
		CA 2042054 A	28-03-1991
		CN 1051058 A	01-05-1991
		DE 69033103 D	17-06-1999
		DE 420358 T	12-10-1991
		ES 2072834 T	01-08-1995
		HU 215179 B	28-10-1998
		IL 95803 A	12-03-1999
		JP 4506007 T	22-10-1992
		LT 1527 A,B	26-06-1995
		LV 10310 A,B	20-10-1994
		WO 9105053 A	18-04-1991
		NO 303988 B	05-10-1998
		PL 167790 B	30-11-1995
		PL 168470 B	29-02-1996
		PT 95447 A,B	22-05-1991
		US 5436156 A	25-07-1995
		US 5863533 A	26-01-1999
WO 9735017 A	25-09-1997	AU 2077197 A	10-10-1997
		AU 2539197 A	10-10-1997
		CA 2248980 A	25-09-1997
		CN 1214081 A	14-04-1999
		EP 0904383 A	31-03-1999
		WO 9735016 A	25-09-1997
		US 5866118 A	02-02-1999
GB 2316082 A	18-02-1998	AU 4118197 A	06-03-1998
		WO 9806856 A	19-02-1998
		EP 0920519 A	09-06-1999
WO 9902668 A	21-01-1999	AU 8379998 A	08-02-1999

Form PCT/ISA/210 (patent family annex) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/EP 99/02311

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9748812 A	24-12-1997	AU 3021697 A	07-01-1998
		CA 2257101 A	24-12-1997
		EP 0904385 A	31-03-1999
		NO 985804 A	10-02-1999
GB 1514156 A	14-06-1978	CH 621686 A	27-02-1981
		AT 353591 B	26-11-1979
		AT 122577 A	15-04-1979
		DE 2704746 A	27-10-1977
		IT 1075113 B	22-04-1985